"FORM PTO-1390 OFFICE (REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER: 524062000201

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 09/936094

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/06001	8 March 2000	8 March 1999

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR TARGETED DRUG DELIVERY

APPLICANT(S) FOR DO/EO/US: Gerhart GRAUPNER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) UNSIGNED.
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: return receipt postcard.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Express Mail Label No.: EL779829475US Date of Deposit: September 7, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Tamara Alcaraz

Tamara Alcaraz

pa 621269

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO PCT/US00/06001	ATTORNEY'S DOCKET NUMBER 524062000201
09/936094		

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provision of PCT Article 33(1)-(4).....$690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....$100.00

CALCULATIONS PTO USE ONLY		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$1,000.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	55 - 20 =	35	x $18.00	$630.00	
Independent claims	9 - 3 =	6	x $80.00	$480.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$0	
TOTAL OF ABOVE CALCULATIONS =				$2,110.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$1,055.00	
SUBTOTAL =				$1,055.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$1,055.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property** +				$	
TOTAL FEES ENCLOSED =				$1,055.00	
				Amount to be refunded:	$0
				charged:	$1,055.00

a. ☐ A check in the amount of $* to cover the above fees is enclosed.

b. ☒ Please charge my **Deposit Account No. 03-1952** (524062000201) in the amount of $1,055.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

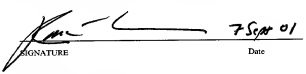
c. ☒ The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to **Deposit Account No. 03-1952**. (524062000201) A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Randolph T. Apple
Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018



SIGNATURE

7 Sept 01
Date

Randolph T. Apple
Registration No. 36,429

PATENT
Docket No. 524062000201

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Tamara Alcaraz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Gerhart GRAUPNER

Serial No.: To Be Assigned

Filing Date: Herewith

For: METHODS AND COMPOSITIONS FOR TARGETED DRUG DELIVERY

Examiner: To Be Assigned

Group Art Unit: To Be Assigned

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The above-referenced application is being filed herewith under 35 U.S.C. § 371.

Applicant wishes to enter the following preliminary amendment to incorporate cross-reference to related applications.

AMENDMENTS

In the Specification:

On page 1, after the title of the invention, please insert the following paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the U.S. National Phase of international application PCT/US00/06001, filed 08 March 2000, which claims priority to U.S. patent application 60/123,352, filed 08 March 1999.--

REMARKS

Applicant makes this Preliminary Amendment prior to examination and without prejudice or disclaimer of any excluded subject matter, and expressly reserves the right to pursue such subject matter in this application or in one or more continuing applications.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 524062000201. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: September 7, 2001 By: _____

Randolph T. Apple
Registration No. 36,429

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Palo Alto, California 94304-1018
Telephone: (650) 813-5933
Facsimile: (650) 494-0792

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 1, after the title, insert:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the U.S. National Phase of international application PCT/US00/06001, filed 08 March 2000, which claims priority to U.S. patent application 60/123,352, filed 08 March 1999.

METHODS AND COMPOSITIONS FOR TARGETED DRUG DELIVERY

Field of The Invention

The field of the invention is targeted drug delivery.

Background of The Invention

Despite a wide variety of drugs, treatment of many diseases still remains problematic due to insufficient specificity of some drugs towards targeted diseased cells. Problems with specificity may further be aggravated by limited transport of the drugs across the cell membrane into the diseased cell. In some cases, drugs will only be active in a particular subcellular location. Several approaches are known in the art to improve drug delivery into a cell.

In one approach, target cells may be subjected to electroporation, during which relatively small pores in biological membranes are created for typically a few milliseconds, thereby allowing an unrestricted influx of molecules into the cell. Electroporation advantageously permits import of drugs into a cell independent of a particular chemical class and/or molecular size. However, electroporation tends to be difficult under *in vivo* conditions, and is not specific towards a particular cell type. Furthermore, electroporation has inherently a relatively high "kill rate" by disrupting organized membrane structures of the treated cells.

In another approach, fusion constructs between a drug and an importer protein are synthesized to increase the uptake of a molecule into a target cell. For example, *Prochiantz* describes the construction of a protein coupled to a homeodomain or homeopeptide derived from a Drosophila transcription factor to translocate peptides across biologic membranes [Prochiantz A. *Ann N Y Acad Sci 1999;886:172-9.* Homeodomain-derived peptides. In and out of the cells], and *Prochiantz* further suggests that the internalization of homeodomains and their fusion constructs into a cell is achieved via formation of inverted micelles. Formation of inverted micelles, however, is generally non-specific towards a particular cell type. Moreover, *Prochiantz's* fusion constructs may be immunogenic and therefore potentially limiting to an *in vitro* environment only. In another example, *Loregian et al.*describe an

intracellular peptide delivery system capable of targeting specific cellular compartments [Loregian A, Papini E, Satin B, Marsden HS, Hirst TR, Palu G. *Proc Natl Acad Sci U S A 1999 Apr 27; 96(9): 5221-6.* Intranuclear delivery of an antiviral peptide mediated by the B subunit of Escherichia coli heat-labile enterotoxin]. Although *Loregian's* chimeric protein is imported into a desired subcellular localization, the delivery is generally not specific towards a particular cell type. Moreover, *Loregian* suggests that the chimeric protein entered through endosomal acidic compartments, a pathway known to endosomal escape and degradation of imported molecules.

To circumvent at least some of the problems associated with degradation, and to increase the specificity of delivery, drugs may be included into a particle. For example, *Tachibana et al.* report [Tachibana R, Harashima H, Shono M, Azumano M, Niwa M, Futaki S, Kiwada H. *Biochem Biophys Res Commun 1998 Oct 20;251(2):538-44.* Intracellular regulation of macromolecules using pH-sensitive liposomes and nuclear localization signal: qualitative and quantitative evaluation of intracellular trafficking] a strategy to target macromolecules to the nucleus via the endocytic pathway. In *Tachibana's* approach, pH sensitive liposomes carry a nuclear translocation signal to direct the liposome to the nucleus. Using pH sensitive liposomes is especially desirable because various problems associated with the endocytotic pathway (*e.g.*, escape, or degradation) are typically circumvented. However, endocytotic transfer is generally not specific towards a particular cell type, thereby limiting *Tachibana's* approach.

In another approach, [Takle GB, Thierry AR, Flynn SM, Peng B, White L, Devonish W, Galbraith RA, Goldberg AR, George ST. *Antisense Nucleic Acid Drug Dev 1997 Jun;7(3):177-85.* Delivery of oligoribonucleotides to human hepatoma cells using cationic lipid particles conjugated to ferric protoporphyrin IX (heme)] *Takle et al.* report an increased target specificity of antisense RNA carrying particles to hepatocytes by packaging the antisense RNA into cationic lipid particles that are loaded with ferric protoporphyrin IX. Despite the cell-specific delivery of antisense RNA to a desired population of target cells, *Takle et al.* did not specifically deliver the antisense RNA to the nucleus, thereby significantly reducing the efficacy of antisense delivery due to cytoplasmatic nucleases and severely limited net concentration of antisense RNA in the nucleus.

In yet another approach, antibodies or antibody fragments are coupled to a drug. The use of antibodies is generally advantageous, because antibodies raised against a particular antigen on the surface of a cell exhibit a high specificity *in vitro* and *in vivo*. For example, *Chen et al.* describe an anti-EGF antibody-poly-L-lysine conjugate to form a gene delivery vehicle [Chen J, Gamou S, Takayanagi A, Ohtake Y, Ohtsubo M, Shimizu N. *Cancer Gene Ther 1998;5(6):357-64* Receptor-mediated gene delivery using the Fab fragments of anti-epidermal growth factor receptor antibodies: improved immunogene approach]. Chen's conjugates selectively delivered a gene to a target cell via (EGFR)-mediated endocytosis, however, the transfer efficiency was only 2% with Fab fragments, and less than 0.1% when whole antibodies were employed. Moreover, antibodies are high affinity binding partners for their respective antigens and will therefore tend to almost irreversibly bind to the antigen of the cell, thereby limiting the efficacy of import to the number of available antigens.

Although various compositions and methods are known in the art to import molecules into a target cell, all or almost all of them suffer from one or more disadvantages. Therefore, there is still a need for improved methods and compositions to specifically deliver drugs to a cell compartment in a target cell.

Summary of the Invention

The present invention is directed to a drug delivery molecule that has a targeting moiety, a routing moiety and a bioactive molecule, wherein the targeting moiety significantly binds to a receptor on the surface of a cell. Binding of the targeting moiety to the receptor (1) does not elicit a significant agonistic effect, and (2) results in an uptake of the drug delivery molecule into the cell. While the routing moiety is coupled to the bioactive molecule, the targeting moiety is coupled either to the routing moiety or to the bioactive molecule.

In one aspect of the inventive subject matter, the receptor is a polypeptide hormone receptor, preferably a somatostatin type II receptor, which is located on a cell. Contemplated cells are endothelial cells proximal to a tumor, and preferred tumors include a lymphoma, mammacarcinoma, an adenocarcinoma, a glioblastoma, and a neuroblastoma.

In another aspect of the inventive subject matter, the targeting moiety is a native or synthetic fragment of the natural ligand of the receptor, or a molecule homologous to a

portion of the natural ligand. Preferred targeting moieties comprise a cyclic peptide. Contemplated routing moieties include a cytoplasmatic retention signal, an endoplasmatic reticulum export signal, a mitochondrial import signal, a nuclear translocation signal, and are preferably coupled to the targeting moiety. It is also contemplated that the coupling is reversible once the drug delivery molecule has entered the cell. The bioactive molecule preferably comprises a drug or prodrug, wherein contemplated drugs may hybridize with a nucleic acid or be a nucleic acid that is expressed in a cell, interfere with (*i.e.*, inhibit or activate) a detoxification process, replication of a cell, inhibit an enzyme or metabolic pathway, or induce apoptosis.

In a further aspect of the inventive subject matter, a method of selectively targeting an endothelial cell proximally located to an anomalous cell has a step in which it is recognized that the endothelial cell proximally located to the anomalous cell has a detectable amount of a somatostatin type II receptor, and that an endothelial cell not proximally located to the anomalous cell is devoid of the detectable amount of the somatostatin type II receptor. In a further step, the endothelial cell proximally located to the anomalous cell is presented with a compound that specifically binds to the somatostatin type II receptor.

Contemplated anomalous cells include neoplastic cells, and especially contemplated anomalous cells are mamma carcinoma cell, a prostate carcinoma cell, and a lung carcinoma cell. Other contemplated cells include ischemically stressed cells and particularly contemplated ischemically stressed cells are retinal pigment epithelial cells, myocytes, cells of the blood brain barrier, and transplanted heterologous cells.

Contemplated compounds comprise a mono- or bi-specific antibody, which may further be coupled to a pharmacological substance, including a thrombin, a radionuclide, or a growth factor, a cytokine, and a nucleic acid. Further contemplated compounds may comprise somatostatin, a somatostatin analog, a fluorophor, a chromophore, a chromogenic substrate, a nucleic acid, and a hapten for a secondary antibody.

In yet other aspects, the presence of detectable amounts of a somatostatin type II receptor on an endothelial cell proximal to a tissue is correlated with a disease of the tissue. In

subsequent steps, compounds are administered to the endothelial cell that specifically bind to the somatostatin type II receptor.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention, along with the accompanying drawing.

Brief Description of The Drawing

Fig. 1A-D are exemplary structures of drug delivery molecules according to the inventive subject matter.

Fig. 2A-E are microphotographs of cells incubated with an exemplary drug delivery molecule according to the inventive subject matter.

Fig. 3 is a graph showing fluorescence intensity in target cells and in control cells incubated with drug delivery molecules according to the inventive subject matter.

Fig. 4 is an immuno-electromicrograph of controls and of choroidal endothelial cells having detectable amounts of somatostatin type II receptors.

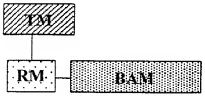
Detailed Description

As used herein, the term "receptor" refers to a structure on the surface of a cell that is at least partially accessible from the outside of the cell, wherein the structure is capable of binding another molecule with high specificity, and wherein binding of the other molecule modulates directly or indirectly a physiological function of the cell by a mechanism other than an increase of a metabolite. The contemplated structure may thereby be a single molecule (typically a polypeptide) with a single binding site, or a multi-component structure in which a plurality of components form a binding site, or in which at least one of the components has a single binding site (*e.g.*, a receptor including modulatory polypeptides without binding function to the other molecule). While contemplated receptors typically have a high binding specificity towards a single molecule, other contemplated receptors may be specific for more than one molecule. For example, a receptor in a neurohormonal signaling pathway may be a multi-component receptor having at least a portion with a hormone binding domain outside of the cell and another portion within the cell coupled to the first portion (*e.g.*, a G-protein) that

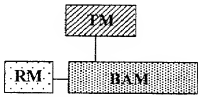
produces a second signal (*e.g.*, cGMP). It should be especially recognized that a transporter on a cell membrane (*e.g.* a glucose transporter) is not regarded as a receptor under the scope of this definition, because a glucose transporter modulates a physiological function (*e.g.*, glycolysis) by an increase of a metabolite.

As also used herein, a molecule that is not identical with the physiological (*i.e.* natural) ligand of a receptor of a cell has a "significant agonistic effect", when binding of the molecule to the receptor elicits a measurable effect on the cell that is at least 25% of a measurable effect of the physiological ligand under comparable conditions. As further used herein, the term "significant binding" means non-covalent binding of two molecules to each other with a dissociation constant K_D of less than about $1x10^{-4}mol^{-1}$. As still further used herein, the term "uptake" refers to any mode of internalization of a molecule into a cell, including receptor-mediated endocytosis, internalization of a receptor-ligand complex, etc.

In **Structures 1A** and **1B**, general structures of contemplated drug delivery molecules are shown and include a targeting moiety TM, a routing moiety RM, and a bioactive molecule BAM.



Structure 1A

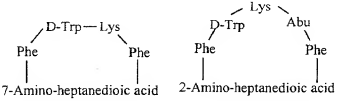


Structure 1B

Contemplated targeting moieties bind significantly to a receptor on a surface of a cell, wherein binding of the targeting moiety to the receptor does not elicit a significant agonistic effect, and wherein binding of the targeting moiety to the receptor results in an uptake of the drug delivery molecule into the cell. A routing moiety is coupled to a bioactive molecule, wherein at least one of the routing moiety and the bioactive molecule is coupled to the targeting moiety.

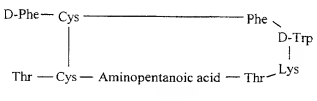
In a preferred aspect of the inventive subject matter, the targeting moiety is a fragment of somatostatin that has no significant agonistic effect. Further preferred targeting moieties include synthetic cyclic or non-cyclic peptides that specifically bind to a somatostatin type II

receptor and examples of preferred targeting moieties having no significant agonistic effect are shown in **Structures 2A-D**.



Structure 2A

Structure 2C



Structure 2D

D-Phe-Cys-Phe-D-Trp-Orn-Abu-Cys-Thr

Structure 2B

Although preferred targeting moieties comprise a fragment or portion of the natural ligand of a receptor (*e.g.*, the somatostatin type II receptor), alternative targeting moieties need not be restricted to portions of the natural ligands of a receptor, and appropriate targeting moieties may include a wide variety of peptides. For example, alternative targeting moieties may include peptides that are homologous to a portion of the natural ligand. Homologous portions may be especially advantageous, where cross-binding with related receptors need to be reduced. Furthermore, where solubility and/or stability of contemplated targeting moieties is a concern, non-proteinogenic amino acids may be included into the targeting moiety. Antibodies, antibody fragments, and antibody-like proteins are not considered a targeting moiety under the scope of this definition.

It should further be appreciated that alternative targeting moieties may also comprise organic and/or organo-metallic compounds, so long as alternative targeting moieties (1) significantly bind to a receptor, (2) binding does not elicit a significant agonistic effect, and (3) binding of the targeting moiety to the receptor results in an uptake of the targeting moiety. Organic and/or organo-metallic compounds may be especially desirable to reduce potential immunogenicity or structural instability while retaining affinity to the target receptor.

Regardless of the chemical composition of contemplated targeting moieties, it is particularly preferred that appropriate targeting moieties include fragments of somatostatin and/or somatostatin analogs which are known to lack agonistic effect. Fragments and analogs lacking an agonistic effect are known in the art [Barrie, R., et al. *J. Surg. Res.* 1993,

55(4):446-450. Inhibition of Angiogenesis by Somatostatin and Somatostatin-like compounds is structurally dependent; Coy *et al.*, U.S. Pat. No. 4,508,711], all of which are incorporated by reference herein. It is even more preferred that appropriate targeting moieties include fragments of somatostatin and/or somatostatin analogs that are known to lack agonistic and antagonistic effect.

It should further be appreciated that the inventive subject matter presented herein is not restricted to a particular type of receptor, and contemplated receptors include hormone receptors, growth factor receptors, neurotransmitter receptors, etc. Especially contemplated receptors include polypeptide hormone receptors, and most preferably a somatostatin type II receptor. Moreover, contemplated receptors need not be limited to a particular structure. Therefore, appropriate receptors may comprise single-subunit and multi-subunit receptors, wherein at least one of the subunits has a binding site or wherein at least two of the subunits form a binding site.

It is preferred that target cells include a microvascular endothelial cell proximally located to a tumor, and particularly contemplated tumors include a mammacarcinoma, an adenocarcinoma, a sarcoma, a lymphoma, a glioblastoma, and a neuroblastoma. The term "endothelial cell proximally located" to a tumor, cell, or tissue refers to an endothelial cell that surrounds, infiltrates or is present within the tumor, cell or tissue. However, in alternative aspects, many target cells other than a microvascular endothelial cell proximally located to a tumor are also contemplated, so long as the cell has a receptor on the surface of the cell that binds specifically to the targeting moiety, and particularly contemplated cells include those that significantly express somatostatin type II receptor on their cell surface, such as pancreatic adenocarcinoma parenchyma and neuroblastoma parenchymatic cells.

In another preferred aspect of the inventive subject matter, the routing moiety comprises a nuclear translocation signal sequence (*e.g.*, KKLK), however, various alternative routing sequences are also contemplated, including a cytoplasmatic retention signal, an endoplasmatic reticulum export signal, and a mitochondrial translocation signal. Where the routing moiety and the targeting moiety are coupled together it is especially contemplated that a carboxyl group from an amino acid of the targeting moiety forms an amide bond with an amino group from an amino acid (*e.g.*, ε-amino group from a Lys) of the routing moiety. It

should be appreciated, however, that many alternative couplings are also contemplated, including covalent and non-covalent coupling. For example, where limited stability of the coupling between the routing moiety and the targeting moiety is desirable, one or more disulfide bonds are contemplated. Disulfide bonds are particularly advantageous, because disulfide bonds are typically stable in an extracellular environment, but readily cleaved within a cell. In another example, a coupling includes a short peptide sequence that is a substrate for an intracellular protease. Such a substrate sequence will specifically be cleaved within a cell and is therefore especially useful in applications where the routing moiety need to be removed from the targeting moiety within the cell. Other non-covalent coupling may include non-peptide couplings, which may be polymers (*e.g.*, functionalized polyethylene glycol), or short bifunctional crosslinker-type molecules (*e.g.*, bismaleimide).

Non-covalent coupling may be particularly advantageous where the stability of the coupling is dependent on the solvent, or other microenvironmental conditions. For example, non-covalent coupling may include charge-complementary coupling (*e.g.*, poly-lysine/poly-glutamate), hydrophobic interaction (*e.g.* leucine zipper), hydrogen bonding (*e.g.*, complementary nucleic acids), or ionic/coordinating interactions (Ni-His complexation), etc.

In a further preferred aspect of the inventive subject matter, the bioactive molecule may comprise a drug or a prodrug, and the term "bioactive molecule" as used herein generally refers to any compound that has a substantial effect on the functioning of a cell, whether as a result of its chemical composition and/or as a result of its isotopic composition. Contemplated bioactive molecules include drugs and prodrugs, and particularly contemplated drugs include a molecule that hybridizes with a nucleic acid, or that interferes with a detoxification process in a cell. Other particularly contemplated drugs inhibit an enzyme, replication of a cell, or induce/prevent apoptosis. For example, molecules hybridizing with a nucleic acid may include sense and antisense DNA, RNA, PNA, and their chemical analogs. Molecules interfering with a detoxification process may include inhibitors of transporters involved in multi-drug resistance. Enzyme inhibitors may include suicide inhibitors, competitive, non-competitive and allosteric inhibitors of hydrolases, ligases, lyases, etc. Furthermore, many drugs are known to inhibit replication of a cell (*e.g.*, α-amanitin) or induce apoptosis (*e.g.*, Fas ligand) all of which are incorporated herein by reference.

In further alternative aspects, the bioactive molecule may also be a non-drug, non-prodrug reporter group that can be used for diagnostic purposes, and contemplated reporter groups include a fluorophor, a radionuclide, a chromophore, and a chromogenic substrate.

With respect to the coupling of the bioactive molecule to the targeting moiety it should be appreciated that the same considerations as for the coupling between the targeting moiety and the routing moiety apply. Furthermore, while it is preferred that the coupling is either between the targeting moiety and the routing moiety, or the targeting moiety and the bioactive molecule, double coupling of the targeting moiety to routing moiety and the bioactive molecule is also contemplated.

It should especially be appreciated that drug delivery molecules according to the inventive subject matter may be employed to deliver a particular drug with high cell and cell compartment specificity. Moreover, contemplated molecules may be utilized to deliver a particular drug specifically to cells that are associated with diseases, in cases where the disease is coupled the presence or overexpression of the receptor being targeted by the targeting moiety. For example, drug delivery molecules according to the inventive subject matter may be employed to inhibit angiogenesis by targeting the somatostatin type II receptor in a drug delivery molecule having a nuclear targeting sequence coupled to an antisense nucleic acid that blocks expression of a vital gene. **Figures 1A-D** show exemplary structures of drug delivery molecules according to the inventive subject matter, wherein BAM is a bioactive molecule.

Contemplated administrations of drug delivery molecules according to the inventive subject matter include *in vivo* and *in vitro* applications of the drug delivery molecule to a target cell. For example, where a particular population of cells is cultured *ex vivo*, the culture medium may be supplemented with the drug delivery molecule. Alternatively, cells may be transiently or permanently transfected with a recombinant nucleic acid to express a target receptor. Such recombinant cells may then be subjected to incubation with the drug delivery molecule. *In vivo* applications may include intravenous injection, oral administration, transdermal application, etc.

It should further be appreciated that preferred drug delivery molecules do not have a significant agonistic effect, and in particularly preferred aspects, the drug delivery molecules do not have a significant agonistic and antagonistic effect due to the particular structure of the targeting moiety.

It should especially be recognized that targeting the somatostatin type II receptor is particularly advantageous because the inventors discovered that endothelial cells that are not proximally located to an anomalous cell do not have detectable amounts of somatostatin type II receptors, whereas endothelial cells that are proximally located to an anomalous cell have detectable amounts of somatostatin type II receptors. As used herein, the term "detectable amounts" refers to amounts of receptors that can be detected by current protocols for immunofluorescence or immuno-electron microscopy.

An example for this discovery is illustrated in Figures 4A-D, showing detection of somatostatin type II receptors in activated microvascular endothelia by silver-enhanced immunogold reactivity in a rat model for laser-induced macular degeneration. Endothelial cells are proximally located within a population of anomalous cells in macular degeneration foci. **Figure 4A** depicts specific antibody staining of choroidal endothelia in a region of neovascularization (anomalous cells are located in laser-treated experimental tissue). Large fusion silver particles indicate clustered gold particles in high density on the luminal surface of endothelial cells, but not on the abluminal surface of endothelial cells. **Figure 4B** shows specific antibody inhibited by specific recognition peptide (laser-treated experimental tissue). No immunoreactivity in either vascular tissue or muscular tissue posterior to choroida (the latter is shown). **Figure 4C** shows specific antibody staining of muscular tissue posterior to choroida (control tissue without laser treatment). No immunoreactivity in endothelial region, but small silver particles at low density demonstrate immunostaining of somatostatinergic neurons in muscular tissue. **Figure 4D** shows a magnified image of microvascular cross-section in a region of neovascularization (same region as shown in A). Large fusion silver particle touching luminal surface of endothelial cell (nucleus, lower right area).

Consequently, it is contemplated that endothelial cells proximally located to an anomalous cell can be selectively targeted, and a contemplated method of selectively targeting an endothelial cell proximally located to an anomalous cell has the following steps:

In one step, it is recognized that the endothelial cell proximally located to the anomalous cell has a detectable amount of a somatostatin type II receptor, and that an endothelial cell not proximally located to the anomalous cell is devoid of the detectable amount of the somatostatin type II receptor. In another step, the endothelial cell proximally located to the anomalous cell is presented with a compound that specifically binds to the somatostatin type II receptor.

In a preferred aspect of the inventive subject matter, the anomalous cell is a neoplastic cell, and particularly contemplated neoplastic cells include a mamma carcinoma cell, a prostate carcinoma cell, and a lung carcinoma cell. Other contemplated anomalous cells include an ischemically stressed cell, for example an ischemically stressed myocyte, cell of a blood brain barrier or a transplanted heterologous cell. However, it is generally contemplated that an "anomalous cell" is a cell that, due to a condition within the cell or within the cell's environment, expresses the somatostatin type II receptor in a detectable amount, whereas the cell would not normally express the somatostatin type II receptor in a detectable amount (*e.g.*, tumor parenchyma).

In further aspects of the inventive subject matter, it is contemplated that the compound that specifically targets the endothelial cell comprises an antibody that specifically binds to the somatostatin type II receptor, wherein the antibody may be mono-specific or bi-specific. Mono-specific or bi-specific antibodies with specificity against the somatostatin type II receptor can be produced by a variety of protocols, and all available protocols are contemplated to be useful for production of contemplated antibodies. Regardless the antibody structure, it is further contemplated that appropriate antibodies may be conjugated with a pharmacologically active substance. For example, where prevention of neovascularisation of a tumor is desirable, the pharmacological substance may be a thrombin that may precipitate clogging of the microvessel. Alternatively, the pharmacological substance may be a radionuclide to specifically direct energy of the radioactive decay of the radionuclide to a desired site (*e.g.*, a tumor). It is still further contemplated that appropriate compounds may also stimulate growth or proliferation of a microvascular endothelial cell. Thus, it is contemplated that the compound may comprise growth factors, cytokines, or a nucleic acid that can be expressed within the microvascular endothelial cell. There are many methods

known in the art to conjugate substances to an antibody, and it is contemplated that such methods are appropriate for use herein.

It should further be appreciated that the method of selectively targeting the endothelial cell may be employed in a diagnostic environment. For example, the method may be employed to determine the presence of an anomalous cell in a specimen. Alternatively, the method of selectively targeting may be utilized to screen for an anomalous cell or cell population. Therefore, it is contemplated that the compound may comprise a reporter group. Many reporter groups are known in the art and it is contemplated that all of the known reporter group can be used in conjunction with the teachings presented herein. For example, where direct access to the endothelia cell is possible the reporter group may be a fluorophor, a chromophore, a hapten for a secondary antibody or a chromogenic substrate. A nucleic acid may be employed as a reporter group where high signal amplification is desired. Where direct access to the endothelia cell is not possible, the reporter group may be a radionuclide or a metal detectable in scanning.

In particularly contemplated aspects of the inventive subject matter, the compound may comprise a drug delivery molecule with a targeting moiety that significantly binds to the somatostatin type II receptor, wherein binding of the targeting moiety to the receptor does not elicit a significant agonistic effect, and wherein binding of the targeting moiety to the receptor results in an uptake of the drug delivery molecule into the cell, and wherein the drug delivery molecule further has a routing moiety and a bioactive molecule, coupled to the routing moiety, wherein at least one of the routing moiety and the bioactive molecule is coupled to the targeting moiety. Alternatively, contemplated compounds may also comprise somatostatin, or a somatostatin analog.

With respect to the step of presenting the endothelial cell proximally located to the anomalous cell with the compound, various methods are contemplated, including direct and indirect methods. Direct methods include incubation of the endothelial cell in a solution containing the compound, while indirect methods include injection of the compound into an organism harboring the endothelial cell proximally located to the anomalous cell.

Anomalous cells can be found in a variety of circumstances. For example, it is known that in some diabetic conditions peripheral circulation is restricted in some tissues due to various causes. As a consequence, malperfused tissue will contain anomalous cells, and the endothelial cells proximally located to the anomalous cells will have somatostatin type II receptors in a detectable amount. Therefore, a method of improving circulation may have one step in which a tissue having a reduced circulation is correlated with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the tissue having the reduced circulation. In another step, a compound is administered to the endothelial cell, wherein the compound specifically binds to the somatostatin type II receptor, and wherein the compound stimulates a cell proliferation of the endothelial cell. It is contemplated that the reduced circulation may be caused by a stenosis of a blood vessel, and it is especially contemplated that the stenosis occurs in a blood vessel located in the brain or heart.

With respect to the compound that stimulates cell proliferation of the endothelial cell it is contemplated that various drug delivery molecules as described above are appropriate so long as such drug delivery molecules have a targeting moiety that binds specific to the somatostatin type II receptor, and so long as the bioactive molecule stimulates the proliferation of the endothelial cell. For example, it is contemplated that appropriate bioactive molecules include cytokines and growth factors.

Furthermore, it is also known that in some vision disorders circulation supporting retinal tissue, and especially the macular region, is restricted due to various causes. Consequently, malperfused tissue will contain anomalous cells, and endothelial cells proximally located to the anomalous cells will have somatostatin type II receptors in a detectable amount. Therefore, a method of improving vision may have one step in which a tissue having a focus of macular degeneration is correlated with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the malperfused tissue. In another step, a compound is administered to the endothelial cell, wherein the compound specifically binds to the somatostatin type II receptor, and wherein the compound inhibits cell proliferation of the endothelial cell. With respect to the compound that inhibits cell proliferation of the endothelial cell, the same considerations as described above apply.

Still further, it is known that in some health conditions, and especially in neoplasms, tumors will be malperfused due to limited nutrient diffusion into the neoplastic tissue. Consequently, neoplastic tissue will contain anomalous cells, and endothelial cells proximally located to the anomalous cells will have somatostatin type II receptors in a detectable amount. Therefore, a method of improving a health condition may have one step in which a tissue having a neoplasm is correlated with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the tissue having the neoplasm. In another step, a compound is administered to the endothelial cell, wherein the compound specifically binds to the somatostatin type II receptor, and wherein the compound inhibits cell proliferation of the endothelial cell.

It is contemplated that the type of neoplasm is not limiting to the inventive subject matter, and appropriate neoplasms may include a lymphoma, a sarcoma, an adenocarcinoma, and a teratocarcinoma. With respect to the compound that inhibits cell proliferation of the endothelial cell, it is contemplated that various drug delivery molecules as described above are appropriate so long as such drug delivery molecules have a targeting moiety that binds specific to the somatostatin type II receptor, and so long as the bioactive molecule inhibits the proliferation of the endothelial cell. For example, it is contemplated that appropriate bioactive molecules may include drugs and prodrugs that may inhibit an enzyme, replication, DNA and RNA synthesis, etc.

Examples

The following examples describe synthesis and an exemplary application of a drug delivery molecule according to the inventive subject matter.

Example 1

Compound I, Compound II, Compound III, and Compound IV were synthesized in a stepwise manner by the Fmoc solid-phase method. Fmoc amino acids, 2-(1 H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 9-Fluorenylmethoxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu), Fmoc-Val-Wang resin, and H-O-*tert*-butyl-L-threonine-2-chlorotrityl resin were obtained from AnaSpec (San Jose, CA) and other reagents for peptide synthesis were from Protein Technologies, Inc. The synthesis was in a 0.025 mmol scale on a Rainin Symphony multiple peptide synthesizer with a 2-(1H-benzotriazol-1-yl)-1,1,3,3-

tetramethyluronium hexafluorophosphate (HBTU)/ n-methylmorpholine activation strategy. Upon completion of the synthesis, the peptides were cleaved from the resin and deprotected (side chain) with TFA containing 2.5% 1,2-ethanedithiol (EDT) and 2.5% H2O. The resin was filtered out from the reaction mixture and the peptides were precipitated with ether.

Routing peptide: KRKLIEENPKKKRKV was synthesized first and then deprotected. Lysine (dissolved in 80% DMF in H_2O) was manually added. The reaction between Texas Red-X succinimidyl ester and the unprotected lysine was allowed to proceed overnight. The rest of the peptide (KKL) was synthesized by the Fmoc solid-phase method.

Compound I: 7-amino-1-carboxy-heptanoic acid (Fmoc-Asu-OH, Fmoc-L-α-aminosuberic acid)FKdWF was synthesized, cleaved and then cyclized by adding 4 equivalents of TBTU and 8 equivalents of diisopropylethylamine (DIEA) for 4h. Cyclized peptide and routing peptide were linked by adding 4 equivalents of TBTU and 8 equivalents of diisopropylethylamine (DIEA) for 4h.

Compound II: ENPKKKRKV was synthesized first and then deprotected. Glu was manually added. The dFCFdW-Orn-Abu-CThr(ol) ClEt-2 resin was used to prepare the fully protected fragment. The EENPKKKRKV-Wang resin and fully protected fragment were joined by adding 4 equivalents of TBTU and 8 equivalents of diisopropylethylamine (DIEA) for 4h. Next, the KRKLI sequence was added to the peptide and then deprotected. Lysine (80% DMF in H2O) was manually added. The reaction between Texas Red-X succinimidyl ester and the unprotected lysine was allowed to proceed overnight. The rest of the peptide KKL was synthesized by the Fmoc solid-phase method.

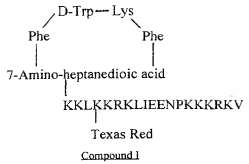
Compound III: Fmoc 6-amino-1-carboxy-heptanoic acid (Fmoc-DL-2-aminoheptanedioic acid was prepared by a H_2N-CH®-COOH + Fmoc-OSu-------> Fmoc-NH-CH®-COOH + O-Su reaction. 6-amino-1-carboxy-heptanoic acid (DL-2-aminoheptanedioic acid)F-Abu-KdWF was synthesized, cleaved and then cyclized by adding 4 equivalents of TBTU and 8 equivalents of diisopropylethylamine (DIEA) for 4h. Cyclized peptide and routing peptide were linked by adding 4 equivalents of TBTU and 8 equivalents of diisopropylethylamine (DIEA) for 4h.

Compound IV: KRKLIEENPKKKRKV was synthesized first and then deprotected. Lysine (dissolved in 80% DMF in H2O) was manually added. The reaction between Texas Red-X succinimidyl ester and the unprotected lysine was allowed to proceed overnight. The rest of the peptide dFCFdWKT-aminopentanoic acid-CTKKL was synthesized by the Fmoc solid-phase method. Synthesis of disulfide-linkage cyclopeptide was done by Cys-oxidation using a dimethyl sulfoxide-mediated oxidation reaction.

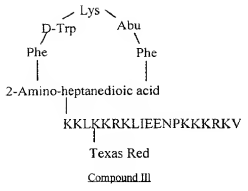
Example 2

Cellular uptake measurements of somatostatin derivatives coupled to a nuclear routing peptide

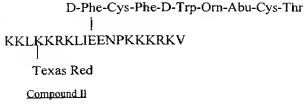
Human neuroblastoma cells (SK-N; SSR2A-positive; (SSR2A=somatostatin type II receptor)) were grown to 50-70% confluency in 25 cm^2-flasks, using DMEM medium supplemented with 1 mM Na-pyruvate, 5 mM non-essential amino acids, and 10% FCS. Hamster CHO cells (SSR2A-negative) were grown under identical conditions. For a typical experiment, cells from one flask were trypsinized, counted in a hematocytometer, seeded as suspension of $1x10^5$ cells/ml onto chamber slides (LabTek single chamber slides #177372, or double chamber slides, or quadruple chamber slides). 24h after seeding, cells were washed 2-3 times with DME and pre-incubated for 30 min with either 1μM somatostatin (Sigma, tissue culture grade) in DME (uptake control condition) or with DME (experimental condition). After removal of the pre-incubation solution, cells were incubated for 2h in either DME containing 1 μM somatostatin plus 1 μM experimental peptide (uptake control condition), or in DME containing 1μM experimental peptide only (experimental condition). Experimental peptides used were compound I, compound II, compound III, compound IV, and routing peptide.



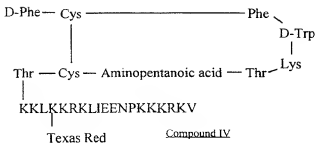
Compound I



Compound III



Compound II



Compound IV

After removal of the incubation solution, cells were rinsed with DME and grown in full medium for additional 30 min, 2h, 24h, 48h, or 72h. The most informative time point of nuclear uptake was determined in initial experiments. Four conditions per experimental peptide were investigated 48h after treatment: Experimental compound with or without somatostatin in neuroblastoma cells, and experimental compound with or without somatostatin in CHO cells.

At times as indicated, cells were rinsed with PBS three times and fixed in 4% paraformaldehyde/PBS overnight at 4-7°C, rinsed with PBS three times and mounted in SlowFade (Molecular Probes).

Confocal analysis was performed with a Zeiss LSM-300 microscope. For each treatment condition, pairs of images (DIC and red channel fluorescence) were taken at 100x oil immersion, with settings for fluorescent signal recording kept constant. Z-sectioning confirmed optimal choice of confocal plane. Images were displayed at identical magnification factors in grayscale mode by Adobe Photoshop software. No signal enhancement or modification procedures were used prior to the measurement of pixel intensities.

As an example, paired images recorded from cells treated with compound IV or routing peptide and grown for additional 48h are presented in Figures 2A to 2E. To evaluate peptide uptake into nuclei proper, nuclei were identified in DIC images, and matching regions in fluorescent images were scanned. Pixels displaying less than 100% black saturation were identified across the nucleus, and their values were recorded and averaged. The pixel intensity averages of at least three nuclei were used to determine the fluorescence intensity of nuclear staining at each experimental condition. Each percent of reduction in black saturation is expressed as 1 relative light unit (*i.e.* reduction to 75% black saturation corresponds to 25 relative fluorescent light units).

Figure 2A illustrates the absence of uptake of compound IV into hamster CHO cells. **Figure 2B** illustrates the absence of uptake of the routing peptide-Texas red conjugate into human neuroblastoma cells, while **Figure 2C** illustrates the absence of uptake of the routing peptide-Texas red conjugate into human neuroblastoma cells in the presence of somatostatin. **Figure 2D** shows the uptake of compound IV into the cell and nuclei of human neuroblastoma cells, and **Figure 2E** shows the uptake of compound IV into the cell and nuclei of human neuroblastoma cells in the presence of somatostatin. In all of Figures 2A-E, the left image represents the DIC photomicrograph and the right image represents the corresponding red channel confocal microphotograph of the same sample.

Relative nuclear fluorescence intensity in human neuroblastoma cells versus hamster CHO cells 48h after treatment

The relative nuclear fluorescence intensity in human neuroblastoma cells versus hamster CHO cells 48h after treatment is shown in **Figure 3**. (Routing peptide (1 μM) in neuroblastoma cells (bar 1), routing peptide (1 μM) plus somatostatin (1μM) in neuroblastoma cells (bar 2), compound IV (1 μM) plus somatostatin (1 μM) in neuroblastoma cells (bar 3), compound IV (1μM) in neuroblastoma cells (bar 4), compound IV (1 μM)co-localizing to the mitotic spindle in neuroblastoma cells (bar 5), compound IV (1 μM) in CHO cells (bar 6). Bar heights represent the mean of pixel intensities. The symmetric error bars reflect the range of variation. 1 relative fluorescence light unit corresponds to a reduction of 1% in black saturation of a standard grayscale.)

Results

Very low level (borderline) nuclear fluorescence was found 30 min after incubation. Specific nuclear fluorescence was increased to significant levels 2h after incubation. Strong levels were detectable through 48h. No significant nuclear fluorescence was detectable 72h after incubation.

In presence of equimolar somatostatin concentrations during the treatment period, no nuclear fluorescence was detectable after 2h. Minimal fluorescence, if any, was detectable after 48h. There was, however, substantial fluorescence associated with the outer cell membrane after 2h, and detectable levels of cytoplasmic fluorescence after48h. The increase of uptake over time suggests that most likely, the very short half life of somatostatin bound to the SSR2A was not sufficient to block entry of compound IV completely; residual compound IV bound to the cellular plasma membrane could have entered the cell through the SSR2A uptake pathway after degradation of somatostatin was complete.

Routing peptide did not display significant staining of the cell surface in the absence of somatostatin; fluorescence intensity was not reduced in the presence of somatostatin. These two observations corroborate the anticipated result that routing peptide by itself has no significant affinity for SSR2A and does not enter neuroblastoma cells by means of an alternative pathway.

In CHO cells, which do not express SSR2A, no significant fluorescence of either nuclei or plasma membrane was found 48h after treatment. The presence or absence of somatostatin in the incubation medium did not affect the fluorescence intensities in CHO cells.

Thus, specific embodiments and applications of methods and compositions for targeted drug delivery have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be

interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

What is claimed is:

1. A drug delivery molecule, comprising:

a targeting moiety that significantly binds to a receptor on a surface of a cell, wherein binding of the targeting moiety to the receptor does not elicit a significant agonistic effect, and wherein binding of the targeting moiety to the receptor results in an uptake of the drug delivery molecule into the cell;

a routing moiety; and

a bioactive molecule, coupled to the routing moiety, wherein at least one of the routing moiety and the bioactive molecule is coupled to the targeting moiety.

2. The drug delivery molecule of claim 1 wherein the receptor is a polypeptide hormone receptor.

3. The drug delivery molecule of claim 1 wherein the polypeptide hormone receptor is a somatostatin type II receptor.

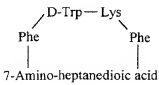
4. The drug delivery molecule of claim 1 wherein the receptor has a natural ligand and the targeting moiety is a fragment of the natural ligand.

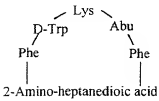
5. The drug delivery molecule of claim 4 wherein the fragment of the natural ligand is synthetic.

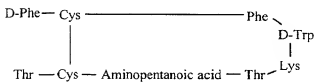
6. The drug delivery molecule of claim 1 wherein the receptor has a natural ligand and the targeting moiety is a molecule homologous to a portion of the natural ligand.

7. The drug delivery molecule of claim 1 wherein the targeting moiety comprises a cyclic peptide.

8. The drug delivery molecule of claim 1 wherein the targeting moiety comprises a molecule selected from the group consisting of:







, and

D-Phe-Cys-Phe-D-Trp-Orn-Abu-Cys-Thr .

9. The drug delivery molecule of claim 1 wherein the cell is a microvascular endothelial cell located proximal to a tumor.

10. The drug delivery molecule of claim 9 wherein the tumor is selected from the group consisting of a mammacarcinoma, an adenocarcinoma, a glioblastoma, a lymphoma, and a neuroblastoma.

11. The drug delivery molecule of claim 1 wherein the routing moiety comprises a moiety selected from the group consisting of a cytoplasmatic retention signal, an endoplasmatic reticulum export signal, a mitochondrial import signal, and a nuclear translocation signal.

12. The drug delivery molecule of claim 1 wherein the bioactive molecule comprises a drug.

13. The drug delivery molecule of claim 1 wherein the bioactive molecule comprises a prodrug.

14. The drug delivery molecule of claim 12 wherein the drug comprises at least one of a molecule that hybridizes with a nucleic acid, and a nucleic acid that is expressed in a cell.

15. The drug delivery molecule of claim 12 wherein the drug comprises a molecule that interferes with a detoxification process in a cell.

16. The drug delivery molecule of claim 12 wherein the drug comprises a molecule that inhibits replication of a cell.

17. The drug delivery molecule of claim 12 wherein the drug comprises a molecule that inhibits an enzyme.

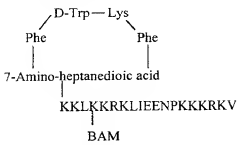
18. The drug delivery molecule of claim 12 wherein the drug comprises a molecule that induces apoptosis.

19. The drug delivery molecule of claim 12 wherein the drug comprises a molecule that inhibits apoptosis.

20. The drug delivery molecule of claim 1 wherein the routing moiety is coupled to the targeting moiety.

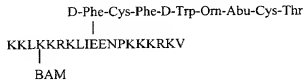
21. The drug delivery molecule of claim 1 wherein the coupling between the targeting moiety and the at least one of the routing moiety and bioactive molecule is uncoupled when the drug delivery molecule is within the cell.

22. A drug delivery molecule having the structure:



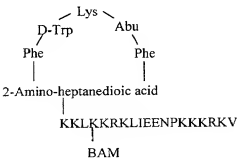
wherein BAM is a bioactive molecule.

23. A drug delivery molecule having the structure:



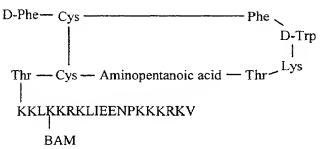
wherein BAM is a bioactive molecule.

24. A drug delivery molecule having the structure:



wherein BAM is a bioactive molecule.

25. A drug delivery molecule having the structure:



wherein BAM is a bioactive molecule.

26. A method of selectively targeting an endothelial cell proximally located to an anomalous cell, comprising:

recognizing that the endothelial cell proximally located to the anomalous cell has a detectable amount of a somatostatin type II receptor, and that an endothelial cell not proximally located to the anomalous cell is devoid of the detectable amount of the somatostatin type II receptor; and

presenting the endothelial cell proximally located to the anomalous cell with a compound that specifically binds to the somatostatin type II receptor.

27. The method of claim 26, wherein the anomalous cell is a neoplastic cell.

28. The method of claim 27, wherein the neoplastic cell is a mamma carcinoma cell.

29. The method of claim 27, wherein the neoplastic cell is a prostate carcinoma cell.

30. The method of claim 27, wherein the neoplastic cell is a lung carcinoma cell.

31. The method of claim 26, wherein the anomalous cell is an ischemically stressed cell.

32. The method of claim 31, wherein the ischemically stressed cell is a cell within a layer of retinal pigment epithelia.

33. The method of claim 31, wherein the ischemically stressed cell is a myocyte.

34. The method of claim 31, wherein the ischemically stressed cell is a member of a plurality of cells that form a blood brain barrier.

35. The method of claim 31, wherein the ischemically stressed cell is a neuron.

36. The method of claim 31, wherein the ischemically stressed cell is a transplanted heterologous cell.

37. The method of claim 26, wherein the compound comprises an antibody.

38. The method of claim 37, wherein the antibody is conjugated with a pharmacologically active substance.

39. The method of claim 38, wherein the pharmacologically active substance comprises a thrombin.

40. The method of claim 38, wherein the pharmacologically active substance comprises a radionuclide.

41. The method of claim 37, wherein the antibody is a bi-specific antibody.

42. The method of claim 26, wherein the compound comprises a growth factor.

43. The method of claim 26, wherein the compound comprises a cytokine.

44. The method of claim 26, wherein the compound comprises a nucleic acid.

45. The method of claim 26, wherein the compound comprises a moiety selected from the group of a fluorophor, a chromophore, a chromogenic substrate, a nucleic acid, and a hapten for a secondary antibody.

46. The method of claim 26, wherein the compound comprises the drug delivery molecule of claim 3.

47. The method of claim 26, wherein the compound comprises a somatostatin.

48. The method of claim 26, wherein the compound comprises a somatostatin analog.

49. The method of claim 26, wherein the step of presenting comprises injection of the compound into an organism harboring the anomalous cell.

50. A method of improving circulation, comprising:

correlating a tissue having a reduced circulation with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the tissue having the reduced circulation; and

administering to the endothelial cell a compound that specifically binds to the somatostatin type II receptor, wherein the compound stimulates a cell proliferation of the endothelial cell.

51. The method of claim 50 wherein the reduced circulation is caused by a stenosis of a blood vessel.

52. The method of claim 51 wherein the stenosis is in a location selected from the group consisting of a brain and a heart.

53. A method of improving vision, comprising:

correlating a tissue having a focus of macular degeneration with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the tissue having the focus of macular degeneration; and

administering to the endothelial cell a compound that specifically binds to the somatostatin type II receptor, wherein the compound inhibits a cell proliferation of the endothelial cell.

54. A method of improving a health condition, comprising:

correlating a tissue having a neoplasm with a detectable amount of a somatostatin type II receptor on an endothelial cell proximally located to the tissue having the neoplasm; and

administering to the endothelial cell a compound that specifically binds to the somatostatin type II receptor, wherein the compound inhibits a cell proliferation of the endothelial cell.

55. The method of claim 54 wherein the neoplasm is selected from the group consisting of a lymphoma, a sarcoma, an adenocarcinoma, and a teratocarcinoma.

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(54) Title: METHODS AND COMPOSITIONS FOR TARGETED DRUG DELIVERY

(57) Abstract

A drug delivery molecule has a targeting moiety, coupled to a routing moiety and a bioactive molecule. The targeting moiety binds to a cell receptor without eliciting an agonistic effect and binding results in uptake of the drug delivery molecule into the cell. Contemplated drug delivery molecules may be employed for treatment and diagnosis. A method of selectively targeting an endothelial cell includes recognizing that presence or absence of somatostatin type II receptors on the endothelial cell correlates with a proximal position of the endothelial cell to an anomalous cell. Endothelial cells proximal to anomalous cells are then presented with a somatostatin type II receptor specific compound. In further methods, the presence of a somatostatin type II receptor on an endothelial cell proximal to a tissue is correlated with a disease of the tissue. In subsequent steps, somatostatin type II receptor specific compounds are administered to the endothelial cell.

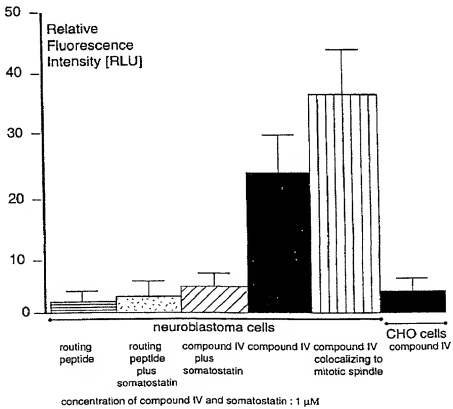

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Figure 1A

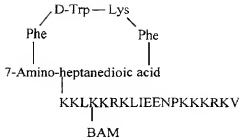


Figure 1B

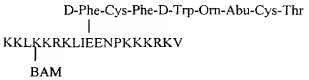


Figure 1C

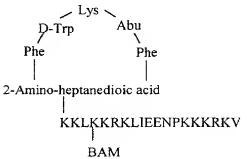
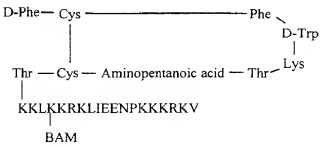
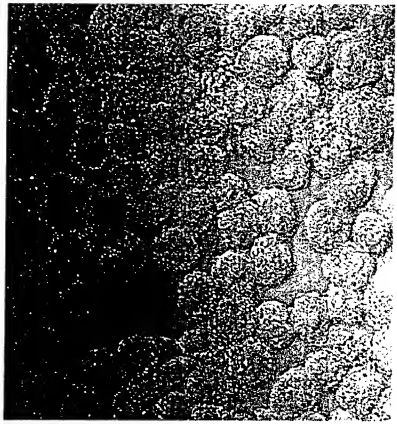


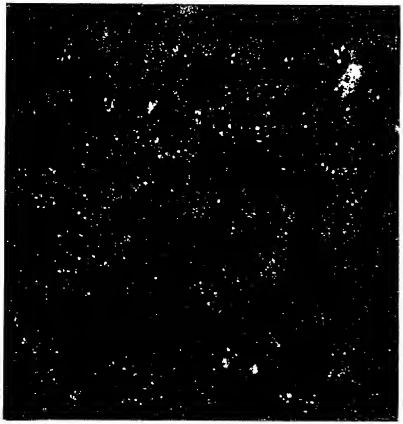
Figure 1D



Absence of uptake of compound IV into hamster CHO cells



DIC image 48h after exposure of CHO cells to compound IV (1 μM)



Red channel confocal image 48h after exposure of CHO cells to compound IV (1 μM)

Figure 2A

Uptake of routing peptide into human neuroblastoma cells
DIC image 48h after uptake of routing peptide (1 µM)
Red channel confocal image 48h after uptake of routing peptide (1 µM)

Figure 2B

Uptake of routing peptide into human neuroblastoma cells in presence of somatostatin

DIC image 48h after uptake of routing peptide (1 µM) in presence of somatostatin (1 µM)

Red channel confocal image 48h after uptake of routing peptide (1 µM) in presence of somatostatin (1 µM)

Figure 2C

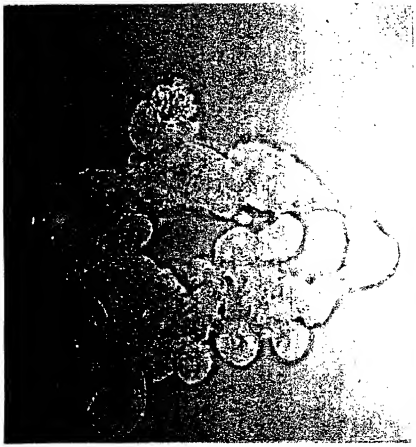
Uptake of compound IV into human neuroblastoma cells

DIC image 48h after uptake of compound IV (1 μM)

Red channel confocal image 48h after uptake of compound IV (1 μM)

Figure 2D

Uptake of compound IV into human neuroblastoma cells:
Interference by oo-incubation with somatostatin



DIC image 48h after uptake of compound IV (1 μM) in presence of somatostatin (1 μM)

Red channel confocal image 48h after uptake of compound IV (1 μM) in presence of somatostatin (1 μM)

Figure 2E

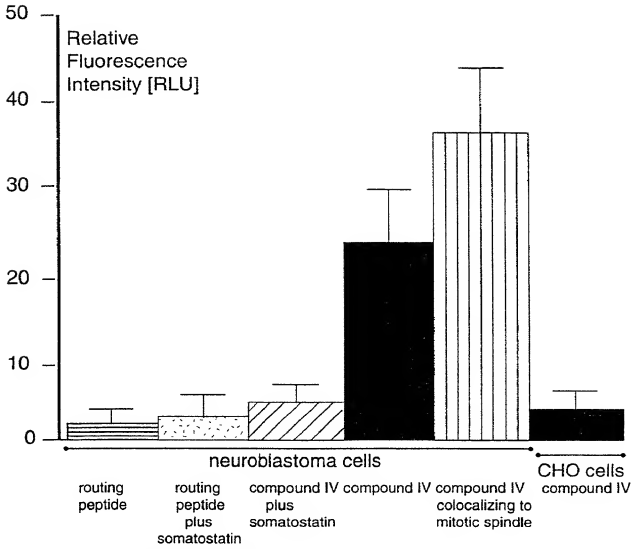
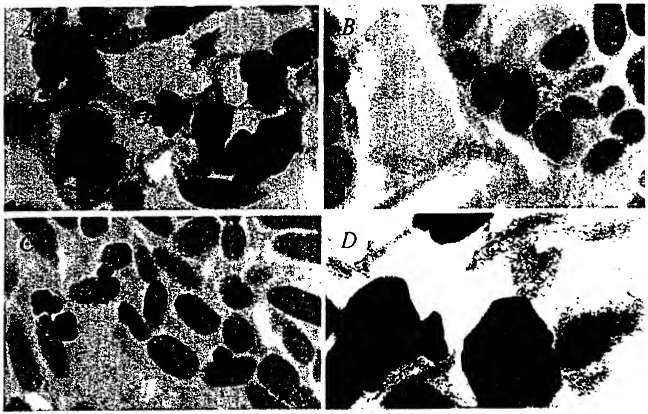

50
40
30
20
10
0
Relative
Fluorescence
Intensity [RLU]
neuroblastoma cells
CHO cells
routing peptide
routing peptide plus somatostatin
compound IV
compound IV plus somatostatin
compound IV colocalizing to mitotic spindle
compound IV
concentration of compound IV and somatostatin : 1 µM

Figure 3

Detection of proliferating microvascular endothelia by immuno-electron microscopy



Magnification: A, B, C 23.000x
D 46.000x

Figure 4

DECLARATION FOR PATENT APPLICATION (Includes Reference to PCT International Applications)	ATTORNEY'S DOCKET NUMBER 524062000201

As a below named inventor I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS AND COMPOSITIONS FOR TARGETED DRUG DELIVERY

the specification of which (check only one item below):

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was filed as United States application

Serial No.
on
and was amended on (if applicable).

☒ was filed as PCT international application

Number PCT/US00/06001
on March 8, 1999

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37 Code of Federal Regulations § 1.56(a) and (b).

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PCT	PCT/US000/06001	March 8, 2000	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
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U S APPLICATION NUMBER	U S FILING DATE	PATENTED	PENDING	ABANDONED
60/123,352	March 8, 1999		*	X

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PCT APPLICATION NUMBER	PCT FILING DATE	U S SERIAL NUMBERS ASSIGNED *(if any)*	PATENTED	PENDING	ABANDONED
PCT/US000/06001	March 8, 2000	*	*	*	X

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Gerhart Graupner		
DATE NOVEMBER 30, 2001	DATE	DATE